Electronic Supplementary Information

Dendrimersomes: a new vesicular nanoplatform for MR-molecular imaging applications

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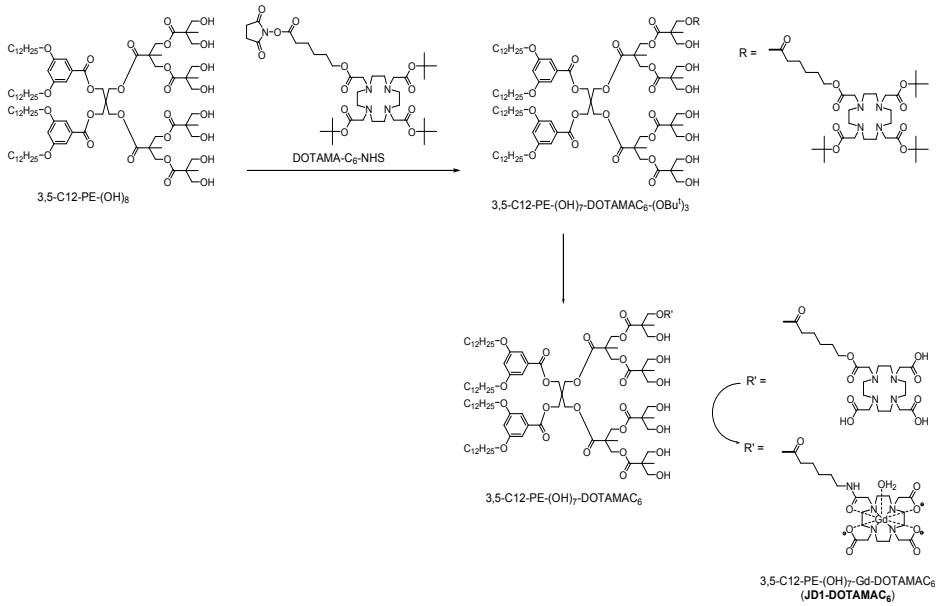
Synthesis Gd-DOTAMAC$_6$-Janus-dendrimer (JD1-GdDOTAMAC$_6$)

Materials and Instrumentation

All chemicals were purchased from Sigma-Aldrich Co. and Alfa Aesar Co. and were used without purification unless otherwise stated. "H_2O" refers to high purity water with conductivity of 0.04 μS cm^{-1}, obtained from a "MILLI-Q" purification system. "Petroleum ether" (PetEt) refers to petroleum ether with boiling point in the range 40-60 °C.
DPTS,[1] isopropyliden-2,2-bis(methoxy)propionic acid,[2] 3,5-(didodecyloxy)benzoic acid[3] and 3,4-(didodecyloxy)benzoic acid[3] were synthesized according to literature procedures.
Thin-layer chromatography (TLC) was carried out on silica plates (silica gel 60 F_{254}, Merck 5554) and visualized by UV lamp (254 nm) or stained in $KMnO_4$ solution. Preparative column chromatography was carried out using silica gel (Merck Silica Gel 60, 230 ± 400 mesh) pre-soaked in the starting eluent. Dialysis tubes (Spectra/Por 7, Spectrum Labs; regenerated cellulose) were soaked in water for 30 minutes and thoroughly rinsed before use. pH measurements were carried out by using a Hanna 211 pH-meter combined with an Aldrich Chemical Company micro-pH electrode, calibrated with buffer solutions at pH 4, 7 and 10. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Eclipse Plus 400 and on a Bruker AvanceIII spectrometers operating at 9.4 and 11.7 T, respectively. Chemical shifts are reported relative to TMS and were referenced using the residual proton solvent resonances. Chemical shifts are reported in ppm and coupling constants in Hz. Splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), double doublet (dd), triplet (t), multiplet (m) or broad multiplet (bm). Electrospray ionization mass spectra (ESI MS) were recorded on a SQD 3100 Mass Detector (Waters), operating in positive or negative ion mode, with 1% v/v HCOOH in methanol as the carrier solvent. MALDI-TOF (Matrix Assisted Laser-Desorption/Ionization Time-Of-Flight) mass spectra were recorded on a Voyager DE-PRO MALDI-TOF (Applied Biosystems, Oggi AB-Sciex, Foster City, California) equipped with a nitrogen laser (337 nm); data acquisition and analysis were performed with a Data-Explorer software, version 4.0.0.0 (Applied Biosystems, Oggi AB-Sciex, Foster City, California); a 3,5-dimethoxycinnamic acid matrix, obtained from LaserBio Labs (Sophia Antipolis Cedex, France), was prepared as 10 mg/ml solution in acetonitrile / ultrapure H_2O / TFA 50:50:0.1%. Infrared (IR) spectra were recorded in the range 4000–400 cm^{-1} at 4 cm^{-1} resolution using a Bruker Equinox 55 spectrometer.

Synthesis Gd-DOTAMAC$_6$-Janus-dendrimer (JD1-GdDOTAMAC$_6$)



Scheme S1. Synthesis of **JD1-DOTAMAC$_6$**

DOTAMA-C_6-NHS

DOTAMA-C_6-NHS was synthesised adapting a literature procedure.[4] In brief, DOTAMA-C_6-OH (1.00 g, 1.34 mmol) and NHS (0.216 g, 1.88 mmol) were dissolved in DCM (5 ml) at 0 °C. A solution of EDC hydrochloride (0.385 g, 2.01 mmol) in DCM (5 ml) was added dropwise, and the resulting mixture was stirred at room temperature for 15 hours. The solution was washed with H_2O (3 x 5 ml), and the organic phase was dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the product was obtained as a white solid (0.97 g, 92% yield). ESI^+ MS, m/z: 783 (M^+), 806 ([M+Na]$^+$). ^{1}H NMR (500 MHz, 25 °C, CD_3OD), δ (ppm): 3.5-3.0 (bm, 8H, NCH_2CO), 3.24 (t, $^3J_{HH}$ = Hz, 2H, $NHCH_2$), 2.85 (s, 4H, *NHS*), 2.65 (t, $^3J_{HH}$ = Hz, 2H, $CH_2COONHS$), 2.5-2.0 (m, 16H, ring), 1.76 (m, 2H, $CH_2CH_2COONHS$), 1.59 (m, 2H, $NHCH_2CH_2$), 1.52 (s, 27H, CH_3), 1.50 (m, 2H, $NHCH_2CH_2CH_2$). ^{13}C NMR (125 MHz, 25 °C, CD_3OD), δ (ppm): 173-168 (CO), 81.4, 81.3 ($Cq^{t\text{-}Bu}$), 56-55 (NCH_2CO), 52.3 (bs, CH_2^{ring}), 38.6 ($NHCH_2$), 30.1 ($CH_2COONHS$), 28.9 ($NHCH_2CH_2$), 27.0 (CH_3), 25.7 ($NHCH_2CH_2CH_2$), 25.1 (CH_2^{NHS}), 24.1 ($CH_2CH_2COONHS$).

3,5-C12-PE-$(OH)_7$-DOTAMAC_6-$(OBu^t)_3$

DOTAMA-C_6-NHS (168 mg, 0.21 mmol) and 3,5-C12-PE-$(OH)_8$ (187 mg, 0.11 mmol) were suspended in dry MeOH (5 ml) and refluxed for 2 days. The solution was evaporated under vacuum and the solid residue was checked by ESI^+ MS, showing to contain the mono-substituted dendrimer. The product was used without further purification. ESI^+ MS, m/z: 1223 ([mono-substituted dendrimer+2H]/2$^+$).

3,5-C12-PE-$(OH)_7$-DOTAMAC_6

3,5-C12-PE-$(OH)_7$-DOTAMAC6-$(OBu^t)_3$ (67 mg) was dissolved in TFA (2 ml) and stirred at room temperature for 15 hours. The solution was evaporated under vacuum and the solid residue was checked by MALDI MS, showing to contain the mono-substituted deprotected dendrimer. The product was used without further purification. MALDI MS, 2279 (mono-substituted deprotected dendrimer).

3,5-C12-PE-$(OH)_7$-Gd-DOTAMAC_6 (JD1-GdDOTAMAC_6)

3,5-C12-PE-$(OH)_7$-DOTAMAC_6 (39 mg) and $GdCl_3$ hexahydrate (17 mg, 0.046 mmol) were dissolved in MeOH (2 ml) and H_2O (2 ml). The pH was adjusted to 6.5 with 1 M NaOH, and the solution was stirred at 50 °C for 15 hours. The mixture was transferred into a dialysis tube (MWCO 1 kDa) and dialyzed vs. 1:1 MeOH/H_2O. After freeze-drying, 15 mg of white solid were obtained and checked by MALDI MS, showing to consist of the mono-substituted Gd-loaded dendrimers. MALDI MS, m/z: 2434 (mono-substituted Gd-loaded dendrimer).

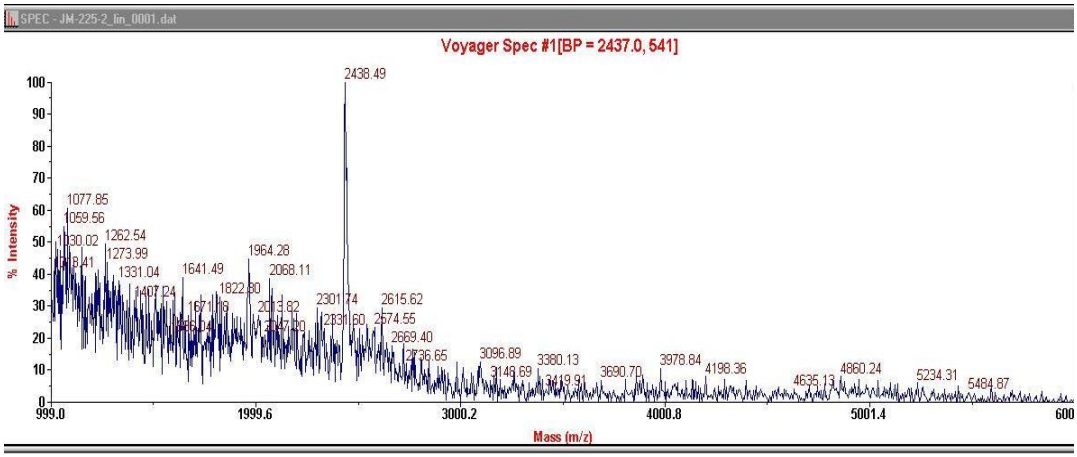


Figure S1. Maldi Mass Spectrum for JD1-GdDOTAMAC_6

Dendrimersomes assembly

Chemicals

While the Janus dendrimers were synthesised according to the protocol described in the previous paragraphs, the anionic lipid 1,2-ditetradecanoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) sodium salt (DMPG) used to assess dendrimersome stability in saline buffers and the 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethyleneglycol)-2000] ammonium salt (DSPE-PEG2000-carboxy) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA) and used as received. The amphiphilic GdDOTAMA($C18)_2$ complex was synthesised according to the procedures previously reported[5]. ProHance® (Gd–HPDO3A) was kindly provided by Bracco Imaging (Milan, Italy). All other chemicals and reagents were purchased from Sigma-Aldrich, and used as received except where noted otherwise.

Solutions

All solutions were prepared by dissolving the required components in double-distilled water, produced with a A4000D Aquatron water still (Stuart, Stone, Staffordshire, UK). All pH measurements were carried out by using a bench pH-meter (Eutech Instruments, Singapore/Oakton Instruments, Vernon Hills, IL, USA) calibrated with buffer solutions at pH 4, 7 and 10 (Merck, Darmstadt, Germany). All osmolarity values were obtained by means of a Type 6 Osmometer (Löser Messtechnik, Berlin, Germany), which calculates the osmolality from the direct measurement on the depression freezing point of aqueous solutions. A basic 0.3 Osm/L and pH 7.4 HEPES enriched saline buffer, simply referred to as *HEPES buffer*, was employed to adjust the osmolarity of all solutions required for the hydration of dendrimeric films and the creation of dendrimersome suspensions. The HEPES buffer was obtained by dissolving the correct amounts of 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES) and NaCl into pure distilled water in order to respect the required concentration for the two components (0.004 M and 0.15 M respectively). The pH was finally adjusted through the addition of NaOH (Sodium Hydroxide Pellets, Carlo Erba, Rodano, Italy) to reach the physiologic value of 7.4. The final osmolarity was checked before the stock solution was stored at 4°C. Moreover, the HEPES buffer was also used to perform the final dialysis necessary to purify all dendrimersome suspensions from the non-encapsulated molecules, and also as hydration solution for dendrimersomes incorporating MRI probes in their bilayer only. For encapsulating dendrimersomes, the hydration required either isosmotic aqueous solutions containing the clinically approved MRI agent Gadoteridol (Gd-HPDO3A, ProHance®) or the water soluble fluorescent probe 5(6)-carboxy-fluorescein (Sigma-Aldrich), in the concentration of 0.25 M and 0.02 M respectively. Both solutions were prepared by adding correct amounts of Gadoteridol or carboxyfluorescein to appropriate volumes of HEPES buffer and distilled water, that were eventually mixed together in order to keep the osmolarity of the final solution precisely to the 0.3 Osm/L value. All the attention devoted to create a perfectly isotonic medium to host dendrimersomes was justified by the perspective that different deformation phenomena on vesicle structure may eventually occur, when nanovesicles experience differences in osmolarity between the internal compartment and the external bulk. Particularly, shrinkage effects or even collapse of liposome geometry have been documented to happen in response to osmotic pressure[6,7,8]. Immediately before use, all solutions were then always checked for their final osmolarity and also for their pH value. For stability tests, some dendrimeric films were hydrated with a 0.3 Osm/L NaCl solution or with a 0.28 M (5% w/w) glucose solution, prepared by dissolving 0.1 g of glucose (Sigma-Aldrich) in 2 ml of simple distilled water, with no salts added.

Dendrimersomes preparation:

Dendrimersomes were obtained by film hydration method.[9] Briefly, appropriate amounts of dendrimers, DSPE-PEG2000 and GdDOTAMA($C_{18})_2$ were accurately weighted to respect the molar ratio provided for each specific kind

of membrane. All required amphiphilic material was deposited as a thin film at the bottom of a 10 mL round bottomed flask, by firstly dissolving it into $CHCl_3$ and then slowly removing the organic solvent through a slow under-vacuum drying process, performed on a rotary evaporator (Rotavapor Heidolph, Schwabach, Germany). During the organic solvent evaporation, a fast rotation was applied to the recipient, in order to make the dendrimer deposition on the glass surface as homogeneous as possible. The film hydration was carried out in a warm bath (Lauda Brinkmann, Delran, New Jersey) at 50 °C for 1 hour, after the addition of the right volume of hydration solution (2 mL) to obtain the desired concentration in the final suspension (10 mg/ml). A vigorous shaking was usually necessary to detach the material from the recipient wall. Depending on the case, films were hydrated with isosmotic aqueous solutions containing Gadoteridol (0.25 M) or carboxy-fluorescein (0.020 M), prepared as mentioned above. In the specific case of dendrimersomes intended to include MRI probes in their membrane only, dendrimeric films were hydrated with HEPES buffer only. To promote the generation of unilamellar vesicles, dendrimersomes were extruded several times through 400 nm and 200 nm pored polycarbonate filters (Lipex extruder, Northern Lipids Inc., Vancouver, Canada) on an 1.5 mL Thermobarrel equipped Lipex Extruder (Nothern Lipids Inc., Vancouver, Canada). The final suspension was inserted into dialysis cellulose tubing membranes (dialysis sacks of 25 mm flat width, Sigma-Aldrich) provided with 12000 daltons sized pores, before an exhaustive dialysis was carried out at 4°C against the isotonic HEPES buffer, in order to purify the dendrimersome suspension from the non-encapsulated probe molecules. Dialysis was always performed in a total volume of at least 2 L and the complete protocol consisted of a first cycle lasting at least 4 h, followed by one buffer renewal and a second longer overnight cycle. However, for fluorescent dye encapsulating dendrimersomes a longer time was spent for dialysis, ranging from one week to ten days with buffer renewal performed each 12 h. In this case, dialysis was intended to be concluded only when the fluorescence intensity measured on the dialysis buffer was negligible. Finally, the vesicles were characterized by Dynamic Light Scattering in order to assess the mean hydrodynamic diameter and the polydispersity of the system.

Dynamic Light Scattering:

Dynamic Light Scattering measurements (DLS) were performed with a Malvern Instruments particle sizer (Zetasizer Nano 90 ZS, Malvern, UK) that uses a scattering angle of 90 degrees to calculate particle size. Specifically, since particles contained in a suspension move due to Brownian motion, they scatter the laser light at different intensities. Therefore, these intensity fluctuations bear an information about the velocity of the movement, that once extracted allows the instrument to convert the measure on the diffusion to size and size distribution, using the Stokes-Einstein relationship. All measurements were performed in triplicate at 298 K in isosmotic filtered (cutoff = 30 nm) HEPES buffer. The Malvern instrument operates a cumulants analysis of the dynamic light scattering intensity autocorrelation function to estimate two values: a width parameter known as the Polydispersity Index (PDI) and a mean value for the size (often referred to as Z-average). This last parameter is a hydrodynamic parameter, that is exclusively calculated from the signal intensity, and must not be intended as a mass or number mean. However, Z-Average (also known as the cumulants mean) can be considered a reliable term to describe the size of particles, but only if the sample is monomodal and monodisperse, and therefore hallmarked by a distribution curve consisting of only one peak with a narrow width. When these conditions are satisfied, Z-Average is the best size indicator produced by DLS, also useful to compare to size values measured with other techniques. Since dendrimersomes prepared by film hydration method presented the qualities mentioned above, their mean hydrodynamic size was expressed through this parameter, being the generally preferred value to report in quality control settings.

The cumulants analysis actually consists of fitting a polynomial to the log of the correlation function (G1).

$$Ln[G1] = a + bt + ct^2 + dt^3 + et^4 + ..$$

where the b value (the second order cumulant) is referred to as the Z-Average diffusion coefficient. With some instrumental constants and the index of viscosity of the dispersant, it contributes to define the mean size parameter (Z-Average).

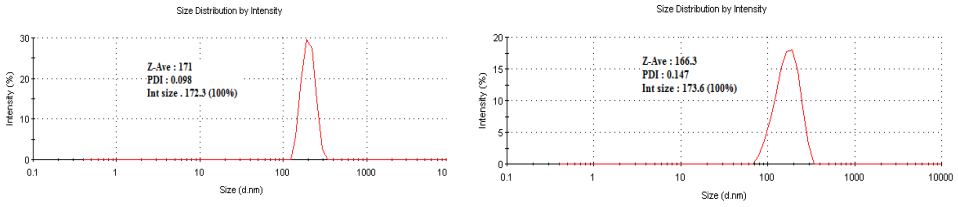
The PDI is algebraically determined as:

$$PDI = \frac{2c}{b^2}$$

The calculations for these parameters are defined in the ISO standard document 13321:1996 E. Specifically, PDI can be intended also as a measure of relative variance on a Gaussian distribution describing a single population of particles characterized by a certain value of Z-Average. Therefore, it can be calculated as ratio of the squares of standard deviation and Z-average size according to the following equation:

$$PDI = \frac{\sigma^2}{Z^2}$$

This Index is scaled such that it normally varies in a range of values included between 0.05 and 0.7: only extremely monodisperse and uniform standards provide values below the first number, while values higher than the second number denote broad size distributions not suitable for DLS analysis. For dendrimersomes, the mean hydrodynamic size (expressed as Z-Average) of most samples was found to be around 160-180 nm with unimodal size distributions, characterized by low values of polydispersity (ranging from 0.09 to 0.19) demonstrating the formation of monodisperse vesicle populations (Figure S2).



Figue (left) or incorporating the JD1-GdDOTAMAC$_6$ into the membrane (right).

Stability in saline buffer:

To prove that dendrimersomes are able to maintain their characteristics in biological relevant media, their stability in glucose and saline solutions recalling physiologic osmotic conditions was tested. Dendrimersomes were firstly prepared by hydration of a film of JD1 dendrimer in 5% w/w glucose solution or in a 0.3 Osm/L NaCl buffer, and then analysed by Dynamic Light Scattering (DLS). The presence of glucose did not affect the ability of JD1 dendrimers to form vesicles with a low PDI (0.095) and a mean hydrodynamic diameter of around 100-120 nm (Figure S3). However, in presence of salts a Z-Average diameter above 1000 nm was observed, revealing that in buffers with salt content comparable to the physiological environment aggregation occurs. Thus, the membrane formulation was adapted to host some charged lipids aiming to induce electrostatic or even steric repulsion forces among particles to avoid aggregation. The addition of the anionic lipid DMPG effectively allowed to obtain a homogeneous suspension of 100-130 nm sized vesicles, but only if the phospholipid was added in relatively large amount (20% in moles). On the other hand, DSPE-PEG-COOH resulted to be effective in producing steric repulsion even in small amounts (5% in moles), thus changing minimally the dendrimeric composition of the membrane. For this reason and for its reported ability to prolong the circulatory lifetime of nanosystems involved in *in vivo* applications, DSPE-PEG2000 was chosen to form a protective coating on dendrimersome surface.

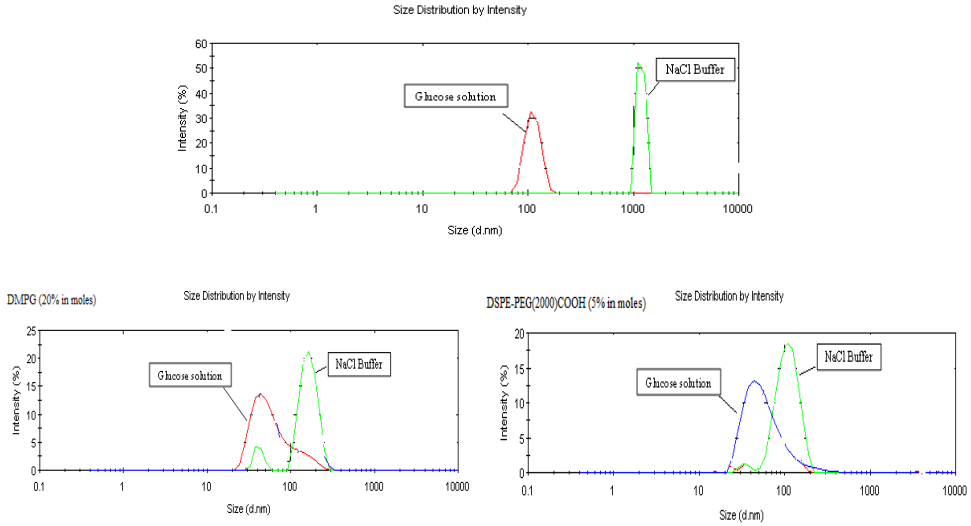


Figure S3. The plots of DLS intensity vs diameter collected from dendrimersomes made of JD1 only (top) and dendrimersomes containing DMPG for 20% in moles (bottom left) or DSPE-PEG(2000) for 5% in moles (bottom right), all prepared in both glucose and NaCl solutions.

^{1}H NMR relaxation measurements

The water proton T_1 relaxation times were obtained on a Stelar SpinMaster Spectrometer (Stelar Snc, Mede, Pavia, Italy) operating at 21.5 MHz, by the standard inversion recovery technique (IR Sequence) requiring 16 experiments of 4 scans. A precise control of the temperature was operated during all measurements by means of a Stelar VTC-91 airflow heater equipped with a calibrated copper–constantan thermocouple (uncertainty of ±0.1°C). Nevertheless, the actual temperature inside the probe head was monitored with a Fluke 52 k/j digital thermometer (Fluke, Zürich, Switzerland) with an uncertainty of ±0.1°C. The first 128 data points of free induction decay were averaged to calculate Magnetization values. To determine r_1 it was necessary to subtract the blank relaxation rate (R_{1dia}) from the observed relaxation rate (R_{1obs}) and then normalize by the Gd^{3+} concentration measured for each sample.

$$R_{1obs} = [CA]r_1 + R_{1dia}$$

$$R_1 = \frac{R_{1obs} - R_{1dia}}{[Gd^{3+}]}$$

R_{1dia} was measured on isosmotic solutions containing dendrimersomes without any paramagnetic complex nor encapsulated neither incorporated.

Specifically, to estimate the concentration of Gd^{3+}, the following equation was applied:

$$[Gd^{3+}] = \frac{R_{1obs} - R_{1dia}}{r_1} \times Dilution\ Factor$$

where R_{1obs} is the relaxivity observed on the sample containing all the ion in a free form, R_{1dia} is the diamagnetic contribution of water and r_1 is the relaxivity of the Gd-aquoion acidic solution. Specifically in acidic conditions R_{1dia} = 0.5 and $r_{1\ (Gd\text{-}aquoion)}$ = 13.5 mM $^{-1}$ s^{-1}, at 20 MHz and 298 K. To apply this equation, the relaxivity R_{1obs} had to be measured on samples, in which the Gd^{3+} was present only in a free form. In order to destroy all dendrimeric material

and completely release the whole amount of paramagnetic ion from vesicles, samples were first diluted in ratio 1:2 (*Dilution Factor*) with 37% pure HCl inside a pre-scored glass ampules. The vials were then spinned in a 5804R Centrifuge (AS instruments Snc, Hamburg, Germany) for 2 minutes at 1000 rpm to collect all the remaining droplets, before being sealed and let in oven all night long at 393 K. All complex was thereby mineralized by the high temperature and the strong acidic conditions, and it eventually released the metal ion. Relaxivity measurement on the sample prepared with this procedure finally entered the equation as R_{1obs}.

$$[Gd^{3+}] = \frac{R_{1obs} - 0.5}{13.5} \times 2$$

^{1}H NMRD profiles

^{1}H NMRD profiles were recorded on a field cycling relaxometer (Stelar Spinmaster FFC 2000; Stelar Snc, Mede, Pavia, Italy) equipped with a silver magnet and able to switch under computer control the magnetic field strength to values of Proton Larmor frequencies ranging from 0.01 to 20 MHz (corresponding to: 2.4×10^{-4} to 0.47 T range). For each field strength, sixteen experiments of two scans each were used for the T_1 determination and each R_{1obs} value was automatically acquired with an absolute uncertainty of ±1%. The typical field sequences used between 8 and 20 MHz was the *non polarized sequence*, while under 8 MHz the *prepolarized sequence* was required. On the other hand, water proton T_1 measurements at a field strength varying from 0.00024 to 1.65 T (corresponding to Proton Larmor frequencies between 20 and 70 MHz) were operated singularly at fixed frequency on a Stelar SpinMaster spectrometer (Stelar Snc, Mede-Pavia, Italy) by means of the inversion−recovery method.

Relaxivity: Gadoteridol encapsulating dendrimersomes

The relaxivity of paramagnetic metal based contrast agents can be affected in different ways by the inclusion in nanovesicular structures. Specifically, the compartmentalization can be responsible for a decreased exchange rate of water molecules between the external environment and the interior space delimited by the membrane.[10] In the case of Gd-HPDO3A (Gadoteridol), the slow crossing of water restricts the efficiency of the contrast agent in shortening relaxation times, in a phenomenon known as quenching of relaxivity. Thus, the release of paramagnetic complexes out of compartmentalized systems is supposed to restore the relaxivity values expected for a free Gd-HPDO3A solution. This effect can be actually achieved by subjecting the vesicle suspension to surfactant addition or by exposing it to intense sonication. A treatment based on the use of the non-ionic surfactant Triton X-100 (a detergent commonly employed to lysate natural membranes) and the mechanical impact of ultrasounds cause the vesicles to lose their membrane properties, leading to the crumbling of the wall structure and to the release of content. In order to assess if a quenching effect on the relaxivity was eventually present in Gadoteridol encapsulating dendrimersomes, a small volume of detergent Triton 100x was diluted in molar ratio 1:100 into the vesicle suspension, and the relaxivity was measured at 21.5 MHz after a cycle of sonication was applied along 90 seconds at about 50 W of power (corresponding to 14 kHz) by means of a Sonopuls ultrasonic tip transducer (Bandelin, Berlin, Germany). Effectively, the vesicle rupture resulted in a slight increase (16.7%) of longitudinal relaxivity to the value ascribed to free molecules of Gadoteridol in water solution at room temperature (4.2 $mM^{-1}s^{-1}$): therefore, a quenching effect of relaxivity might be present but only to a very limited extent (Figure S4).

Moreover, when the temperature increases, the texture assembled by JD1 dendrimers fluidizes, eliciting a totally free conveyance of water molecules and restoring therefore any loss of relaxivity detected at room temperature. In fact, at 310 K intact dendrimersomes recalled very closely the expected relaxivity values for a simple Gadoteridol aqueous solution at this temperature (3.58 $mM^{-1}s^{-1}$ at 21.5 MHz), meaning that the relaxivity limitation caused by the membrane is removed because of the enhanced membrane permeability and the accelerated water exchange (Figure S4).[11] As confirmation of the fact that the higher temperature does not correspond to an r_1 decrease (as it is expected for free Gadoteridol), the whole ^{1}H NMRD profile of Gadoteridol encapsulating dendrimersomes acquired at 310 K

results to be almost coincident with that measured at 298 K (Figure S5).

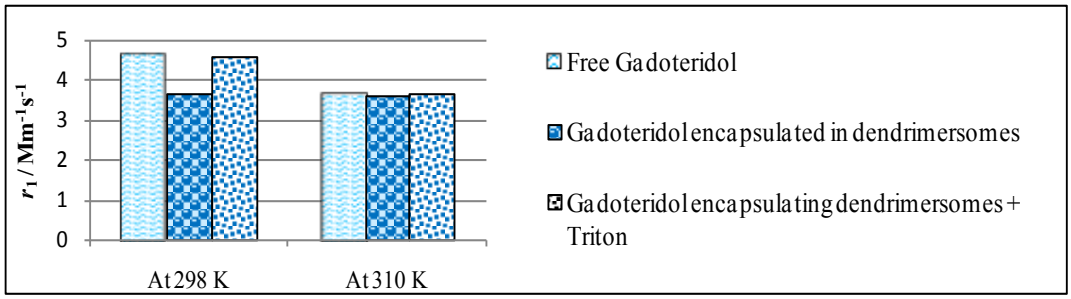


Figure S4. Relaxivity at 21.5 MHz of Gadoteridol in a free form, entrapped into the inner aqueous core of dendrimersomes or dispersed into the solution after Triton 1x addition and intense sonication, measured at 298 K (left) and 310 K (right).

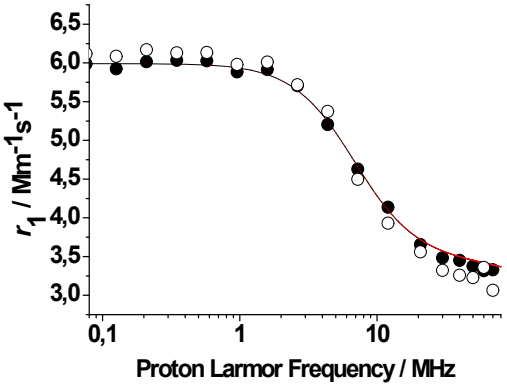


Figure S5. . ^{1}H NMRD profiles of Gadoteridol encapsulating dendrimersomes acquired at 298 K (●) and at 310 K (○). The line refers to the fitting of the profile collected at 298 K.

Relaxivity: Paramagnetic complex incorporating dendrimersomes

The relaxivity of JD1-GdDOTAMAC$_6$ and GdDOTAMA(C$_{18}$)$_2$ incorporating dendrimersomes was measured also at 310 K (21.5 MHz). The observed higher r_1 values (Figure S6) are commonly expected for GdDOTAMA-based systems, and they are easily explained by the acceleration of water exchange rate on the paramagnetic center. However, the increased water permeability across the membrane might play an additive role in enhancing the relaxivity, since it facilitates the water molecules to reach the Gd-chelates exposed on the interior surface of vesicles.

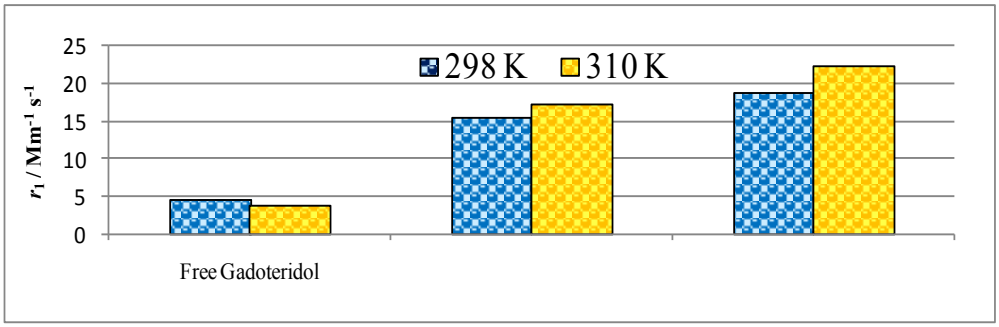


Figure S6. Relaxivity at 21.5 MHz of Gadoteridol in a free form and dendrimersomes incorporating JD1-GdDOTAMAC$_6$ and GdDOTAMA(C$_{18}$)$_2$ into the membrane measured at 298 K and 310 K.

Fluorescence

In order to test if dendrimersomes were able to encapsulate also fluorescent probes in the inner aqueous core, vesicles internalizing the water soluble fluorescent probe 5(6)-carboxyfuorescein (Sigma-Aldrich) were prepared by simply hydrating the thin film with a 300 mOsm fluorescent dye aqueous solution. When the fluorescent probe is highly concentrated, an extinguishing effect of fluorescence intensity (named *fluorescence self-quenching*) is expected to occur.[11,12] This light emission drop is attributable to the contact and to the consequent non radiative transfer of energy among adjacent dye molecules.[11,12] Since the carboxyfluorescein solution used for dendrimeric film hydration was highly concentrated (0.02 M), dendrimersomes were not expected to produce a strong light emission. However, because relying on the concentration of carboxyfluorescein, an increase of fluorescence signal is supposed to occur in case of vesicles opening, leading to probe release.

To detect fluorescence intensity variations associated with the release of fluorescent probe from dendrimersomes, a FluoroMax-4 Spectrofluorometer (Horiba Scientific, Edison New Jersey, USA) equipped with the driving software FluorEssence™ for Windows was used. The excitation and emission monochromators were set to select a band of wavelengths centered on the carboxyfluorescein specific excitation and emission wavelengths (492 and 517 nm respectively) and fluorescence spectra were acquired. The experiment consisted of two measurements, one of which was intended to assess the fluorescence intensity on the sample containing intact dendrimersomes, while the other one was intended to evaluate fluorescence intensity after having subjected dendrimersomes to Triton 1x mediated lysis and sonication. The former measurement was expected to produce low values of emitted light intensity, while the latter case was supposed to detect a signal intensity increase due to the release of carboxyfluorescein probe.

To perform the fluorescence measurement, the sample was first diluted into isosmotic HEPES buffer in ratio 1:100 (specifically by mixing 30 uL of sample and 2.97 mL of buffer) and then inserted into the appropriate transparent cuvette, before the emission spectrum representing the fluorescence intensity emitted from intact dendrimersomes was acquired. On the other hand, to induce a complete rupture of vesicles, 30 uL of Triton 100x detergent were added to 2.94 mL of HEPES buffer and 30 uL of sample, to reach a final volume of 3 mL where all dilution proportions were respected. An intense cycle of sonication (14 kHz) was still applied along 90 seconds before the emission spectrum was acquired. As expected, a five fold increased fluorescence intensity was observed in correspondence to the carboxyfluorescein emission wavelength after the vesicle suspension underwent the detergent and sonication treatment. (Figure S7).

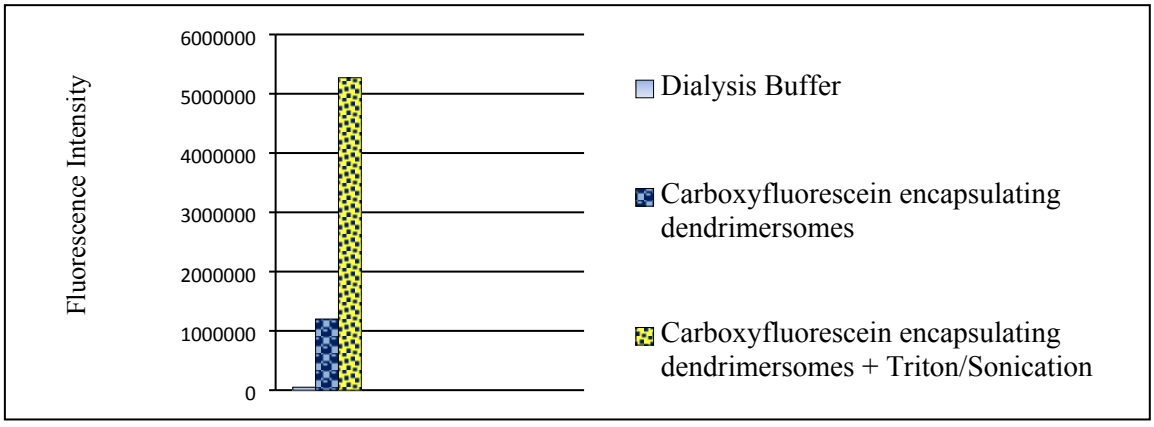


Figure S7. Fluorescence intensity (arbitrary units) measured at 517 nm on dialysis buffer, on a suspension of carboxyfluorescein encapsulating dendrimersomes and on the same suspension after Triton 1x addition and ultrasound exposition.

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